Ultrashort NSAID-conjugated Peptides as Bifunctional Nanomaterials

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Biofunctional Nanomaterials Group

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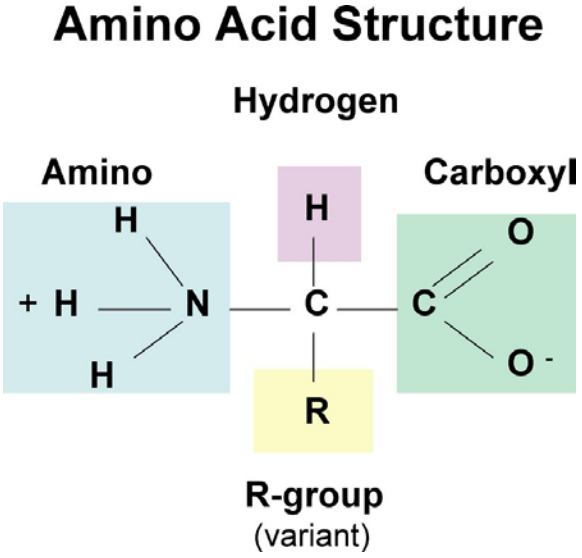
Outline

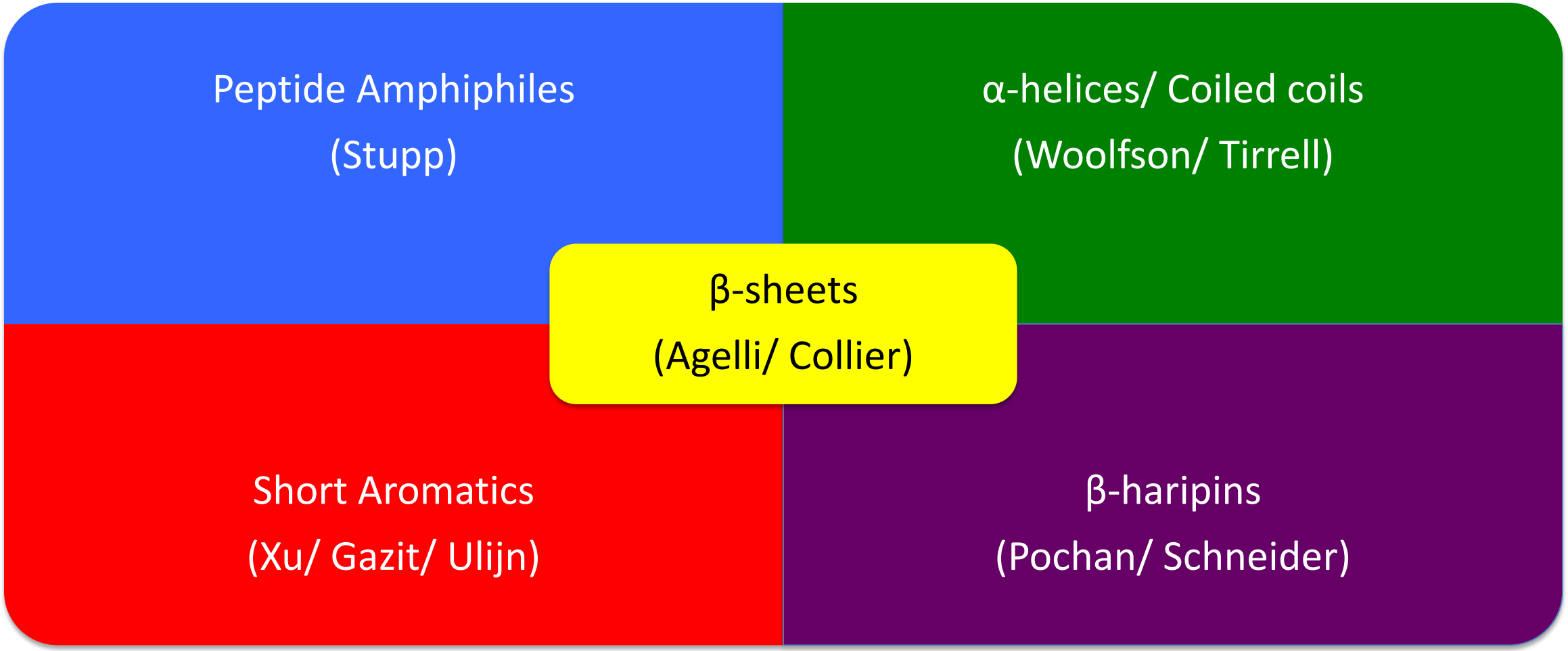
1. Ultrashort peptides
2. Self-assembling peptides
3. Ultrashort self-assembling antimicrobial peptides
4. NSAID-conjugated self-assembling peptides

What are Ultrashort Peptides?

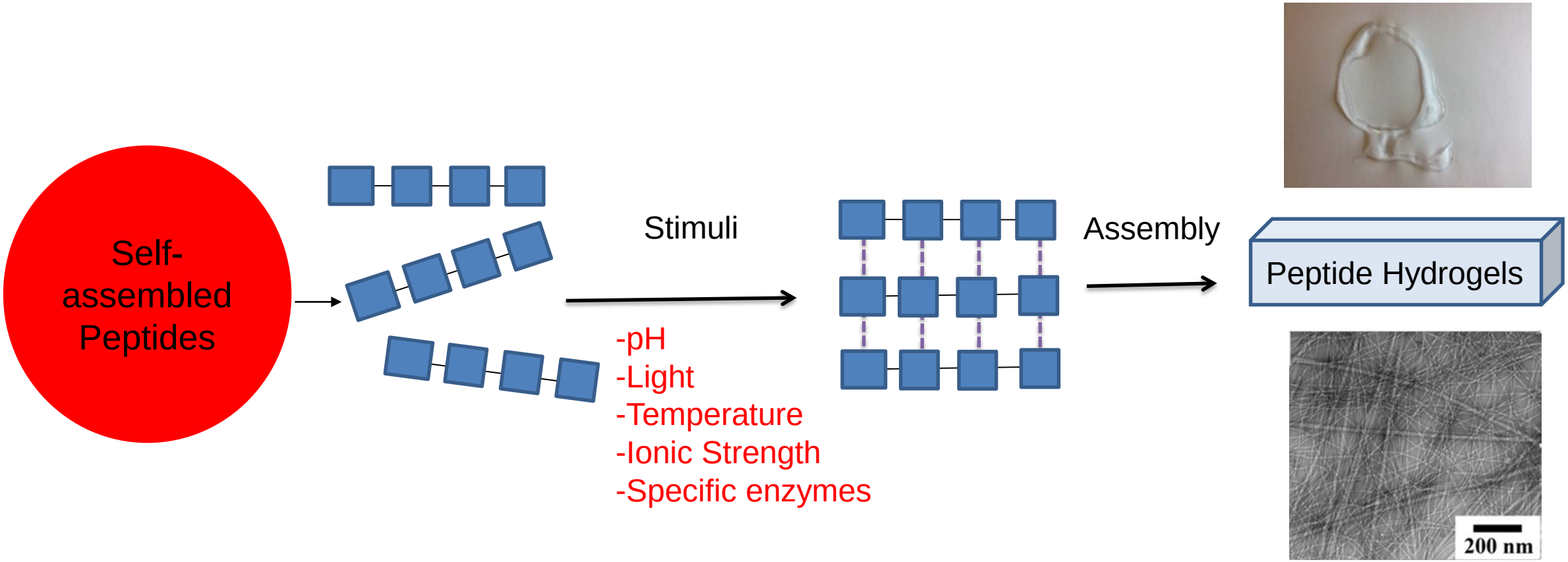
- Ultrashort= 4-7 amino acids
- Cationic= net positive charge (+2)
- Cost effective → Upscale → Translational potential → Patient
- Numerous advantages including:
  - ↑chemical versatility
  - ↓immunogenicity
  - Tunable biocompatibility + biodegradability
  - Tailored self-assembly/pharmacological properties
  - Antimicrobial= innate immune response
  - Nanotechnology



Self-assembling Peptides



Core Technology



Short peptide sequences

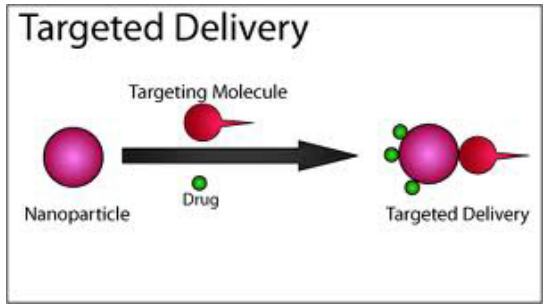
External stimuli

Hydrophobic: Hydrophilic ➔Hydrogel
(critical gelation concentration)

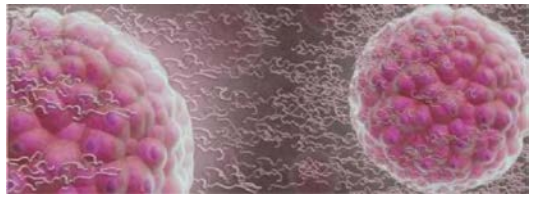
Biofunctional Nanomaterials Utilising the Building Blocks of Life!

Infection and Medical Devices

Wound healing



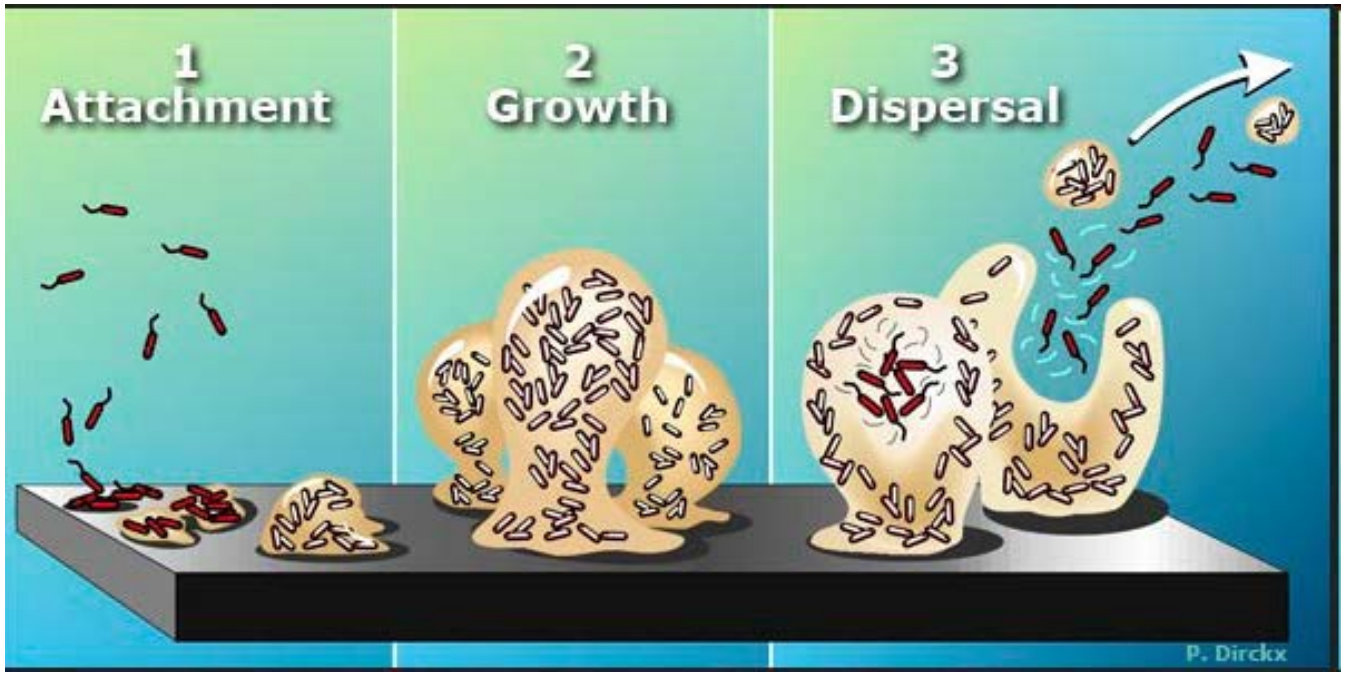
Drug Delivery



Stem Cells/Regenerative medicine

Planktonic vs. Biofilm Bacteria

- Planktonic form: Free floating in liquid
- Biofilm form: sessile, composed of aggregated microcolonies of cells surrounded by a protective extracellular polymeric matrix
- Mature biofilms can resist 10-1000 times the concentrations of standard antibiotic regimens that are required to kill genetically equivalent planktonic forms



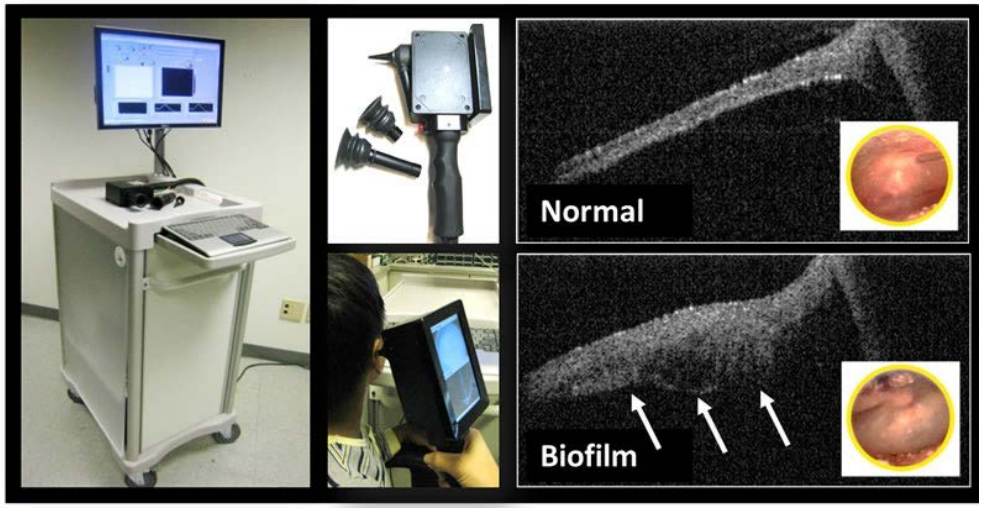
P. Dirckx, Centre for Biofilm Engineering, Montana State University, Bozeman

Biofilms and Implant-Associated Infections. Laverty, G., Gorman, S.P. and Gilmore, B.F. In: Biomaterials and Medical Device Associated Infections. Woodhead Publishing Ltd. 2014.

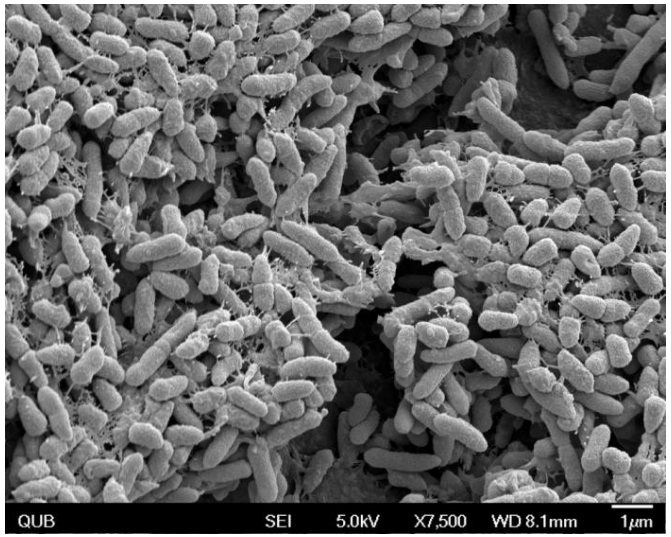
Biofilms in the Environment and Medicine

Biofilm growth on rocks in a stream (USGS) and within a kitchen pipe (MSU Center for Biofilm Engineering).

Biofilm formation on a voice prosthesis implant.



University of Illinois researchers tested a prototype of a new device that can see biofilms behind the eardrum to better diagnose and treat chronic ear infections.



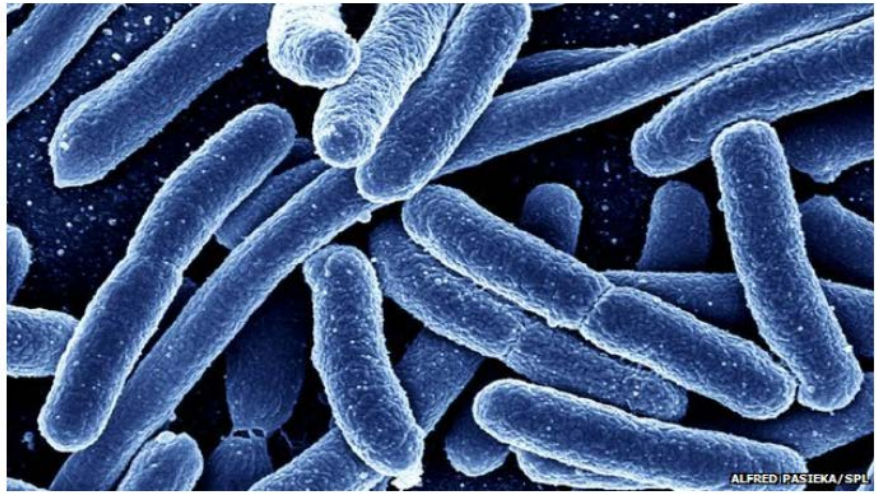
SEM Pseudomonas aeruginosa, shown here attached to an implant surface, is one of many resistant microorganisms

Antimicrobial Resistance

BBC NEWS

Superbugs to kill 'more than cancer' by 2050

COMMENTS (565)



Drug resistant E.coli bacteria are already a significant problem in Europe

Drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer - by 2050 unless action is taken, a study says.

They are currently implicated in 700,000 deaths each year.

Related Stories

Analysis: Antibiotic apocalypse

- Healthcare associated infections
- Medical devices: reservoir for “superbugs”
- Chronic wounds
- Persistent burden on:
  - Patient morbidity & mortality
  - Family and carers
  - Healthcare budgets

sky NEWS

Superbugs 'Could Send UK Back To The Dark Ages'

Action is needed to stop the world entering a post-antibiotic era in which common infections and injuries can kill, say experts.

17:49, UK,
Wednesday 02 July 2014

What are the solutions?

Chem Biol Drug Des 2010; **75:** 563–569
Research Article

doi: 10.1111/j.1747-0285.2010.00973.x

Antimicrobial Activity of Short, Synthetic Cationic Lipopeptides

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Biomaterials Research Group, School of Pharmacy, Queens University of Belfast, Medical Biology Centre, 97 Lisburn Road

for new antimicrobial agents with activity against pathogens that are resistant to the available armoury of antibiotics (3,4).

One class of compounds that has attracted increasing attention in the last two decades are the cationic antimicrobial peptides (AMPs). Antimicrobial peptides are short (typically ranging from 12

Pathogens **2014**, *3*, 791-821; doi:10.3390/pathogens3040791

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Review

Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications

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BioMACROMOLECULES

Article

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Ultrashort Cationic Naphthalene Derived Self-Assembled Peptides As Antimicrobial Nanomaterials

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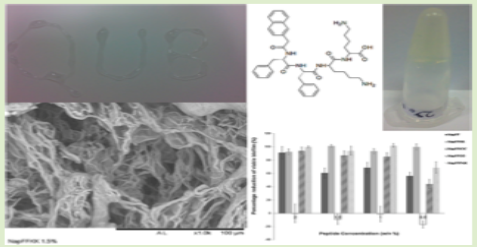
Supporting Information

ABSTRACT: Self-assembling dipeptides conjugated to naphthalene show considerable promise as nanomaterial structures, biomaterials, and drug delivery devices. Biomaterial infections are responsible for high rates of patient mortality and morbidity. The presence of biofilm bacteria, which thrive on implant surfaces, are a huge burden on healthcare budgets, as they are highly resistant to current therapeutic strategies. Ultrashort cationic self-assembled peptides represent a highly innovative and cost-effective strategy to form antibacterial nanomaterials. Lysine conjugated variants display the greatest potency with 2% w/v NapFFKK hydrogels significantly reducing the viable *Staphylococcus epidermidis* biofilm by 94%. Reducing the size of the R-group methylene chain on cationic moieties resulted in reduction of antibiofilm activity. The primary amine of the protruding R-group tail may not be as readily available to interact with negatively charged bacterial membranes. Cryo-SEM, FTIR, CD spectroscopy, and oscillatory rheology provided evidence of supramolecular hydrogel formation at physiological pH (pH 7.4). Cytotoxicity assays against murine fibroblast (NCTC 929) cell lines confirmed the gels possessed reduced cytotoxicity relative to bacterial cells, with limited hemolysis upon exposure to equine erythrocytes. The results presented in this paper highlight the significant potential of ultrashort cationic naphthalene peptides as future biomaterials.



Research Article SOJ Microbiology & Infectious Diseases

Cationic Antimicrobial Peptide Cytotoxicity

Garry Laverty* and Brendan Gilmore

Research article

Journal of PeptideScience

Received: 13 May 2015 Revised: 26 June 2015 Accepted: 6 July 2015 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/psc.2805

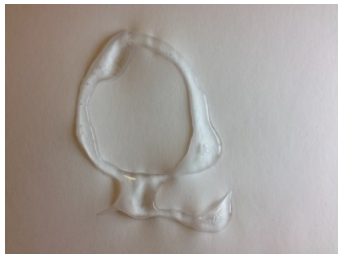
Anti-biofilm activity of ultrashort cinnamic acid peptide derivatives against medical device-related pathogens

Garry Laverty,* Alice P. McCloskey, Sean P. Gorman and Brendan F. Gilmore

Rational Design of Antimicrobial Peptide Motif vs Self-assembly

Antimicrobial Activity	Propensity to Self-assemble
Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)	Hydrophobic/Hydrophilic balance
Interactions with microbial extracelluar membranes	Non Covalent intermolecular interactions (e.g. Van der Waal's, π-π stacking)
Interaction with intracellular targets/processes (DNA, RNA, enzymes, protein synthesis)	Ability of peptide to form hydrogen bonds with each other and with water

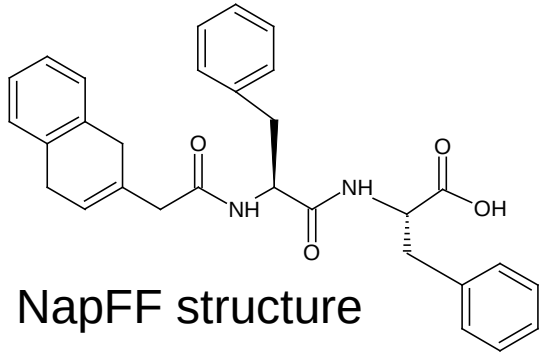
McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.



Self-assembled Ultrashort Peptide Gels

- Successful library of ultrashort peptides: self-assembled at physiological pH
- (X_1-FF-X_2)
- Hydrophobicity: naphthalene (Nap) grouping (at X_1) and varying quantity of phenylalanine (F) in primary structure
- Minimum of 2 charged units required for antimicrobial activity
- Primary amine group provides cationic charge
- Cationic amino acids vary by number of methylene units on R-group

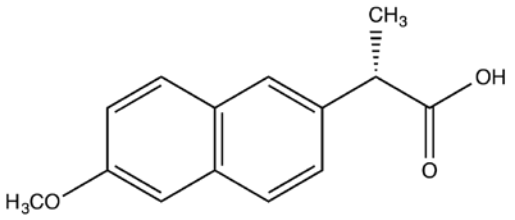
-Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.



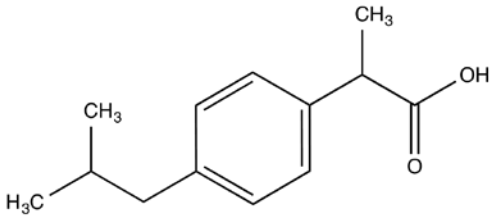
NapFF structure

Dual Antimicrobial Anti-inflammatory Nanomaterials

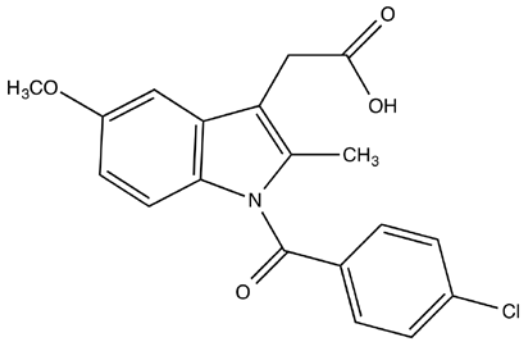
- Hydrophobicity provided by NSAID structure
- High in aromaticity
- Display self-assembly and gelation characteristics
- Potential applications in chronic infected wounds



Naproxen



Ibuprofen



Indomethacin

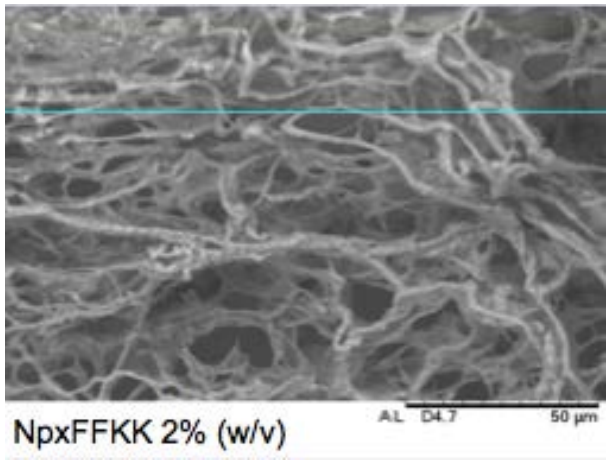
A.P. McCloskey, S.M. Gilmore, J. Zhou, L. Chen, E.R. Draper, B.F. Gilmore, D. Adams, B. Xu, G. Laverty*. Self-assembling Ultrashort NSAID Peptidomimetics: Bifunctional Antimicrobial and Anti-inflammatory Nanomaterials. *Adv.Healthc.Mater*. *In preparation.*

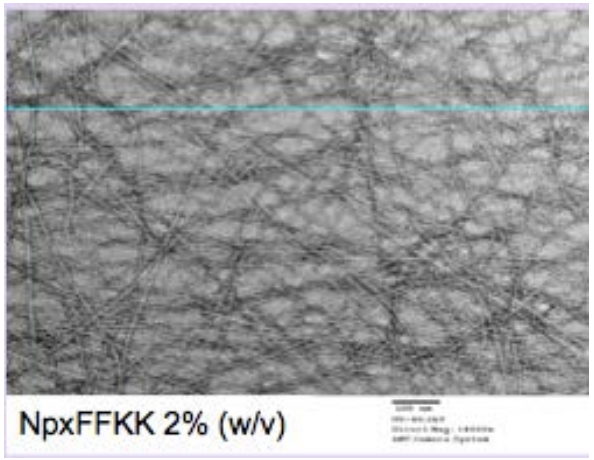
Self-assemble to Hydrogel Networks

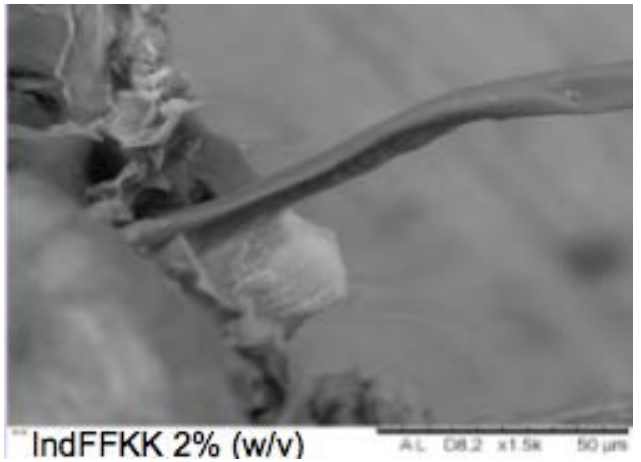
NpxFFKK 0.5 %

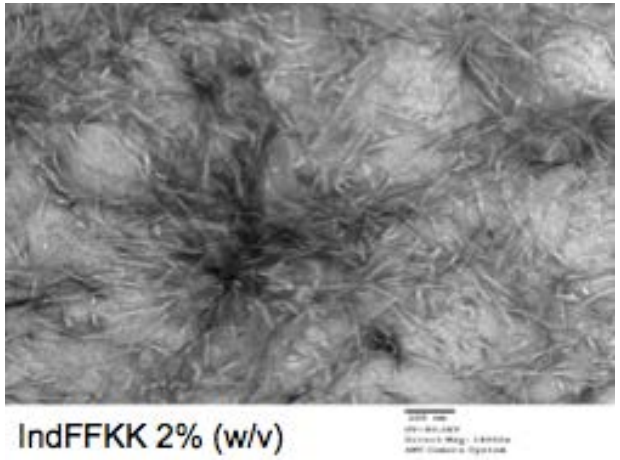
IbuFFKK 2 %

IndFFKK 1 %









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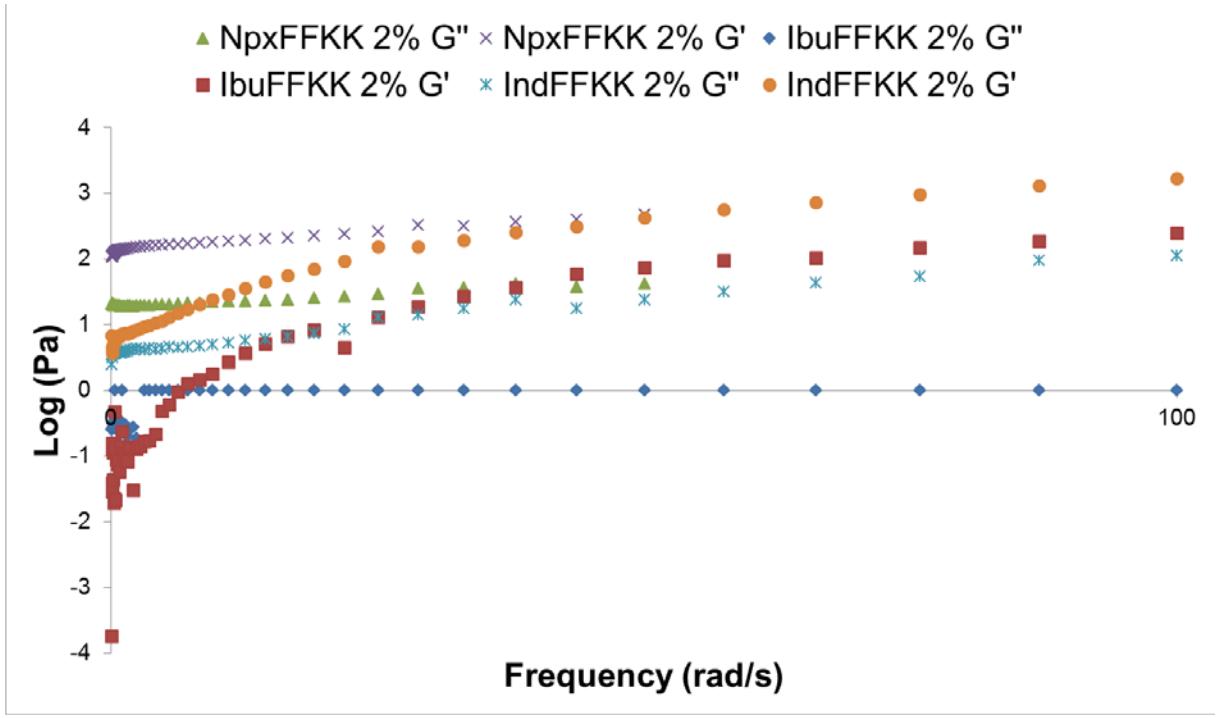
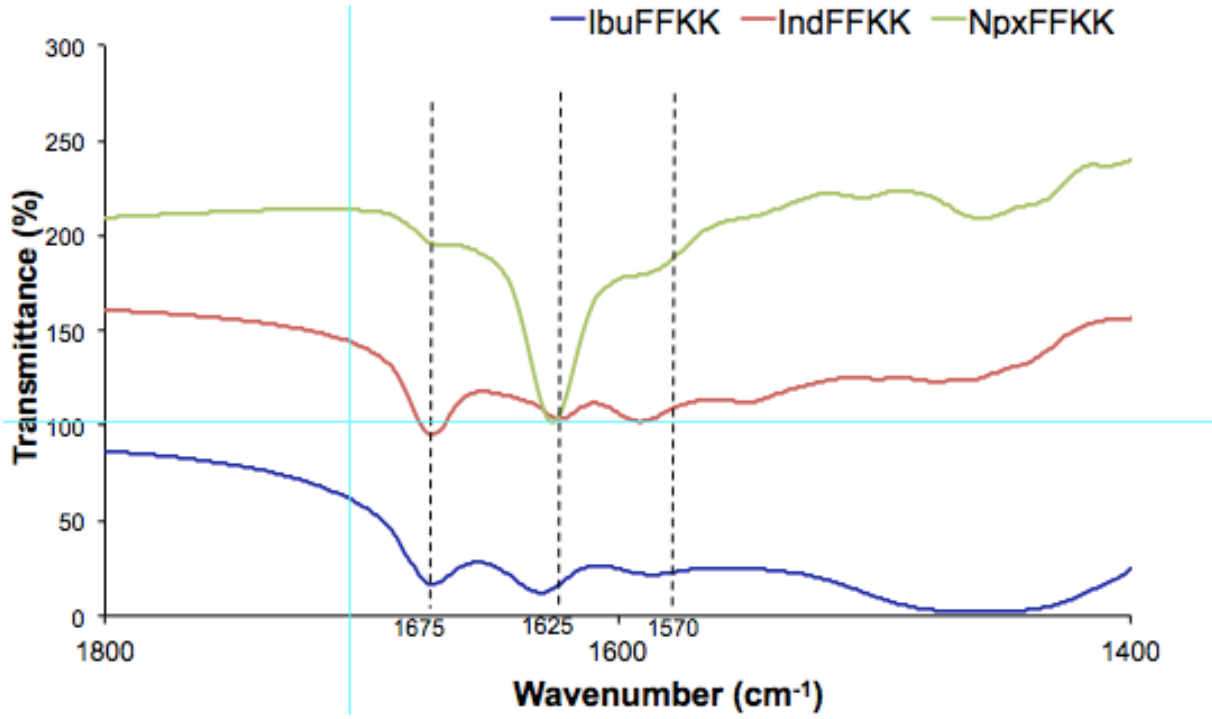
Brandeis

Confirmation of β-sheet Hydrogel Networks



Oscillatory rheology



FTIR

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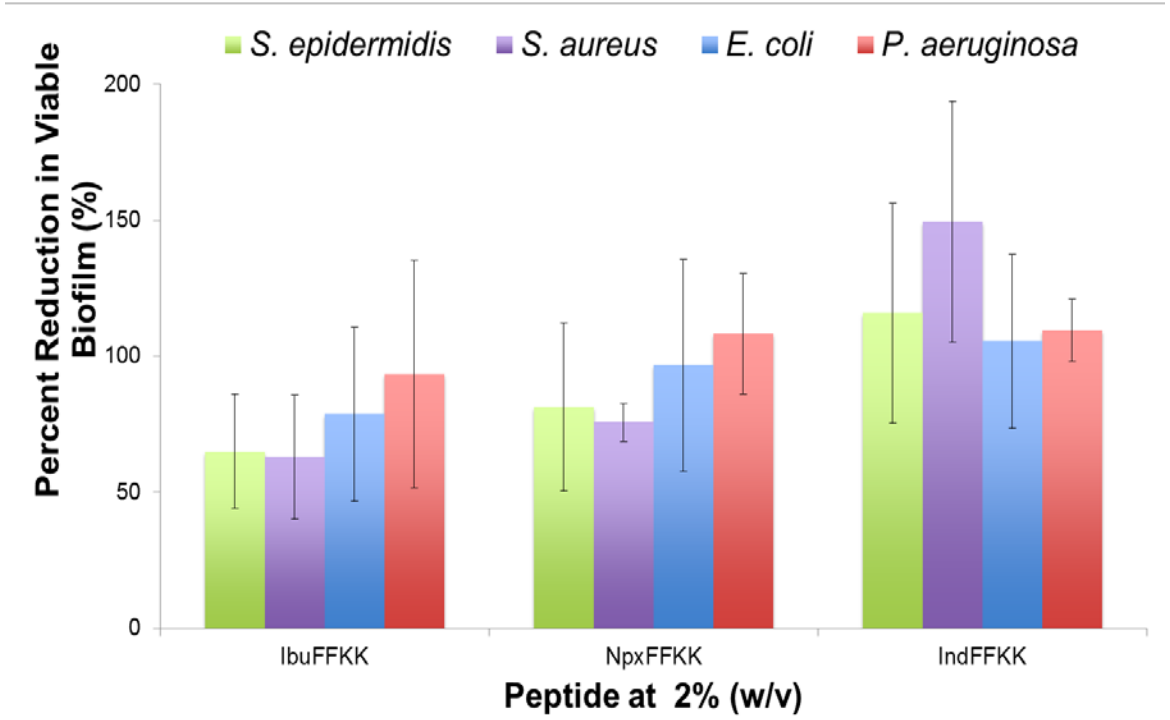
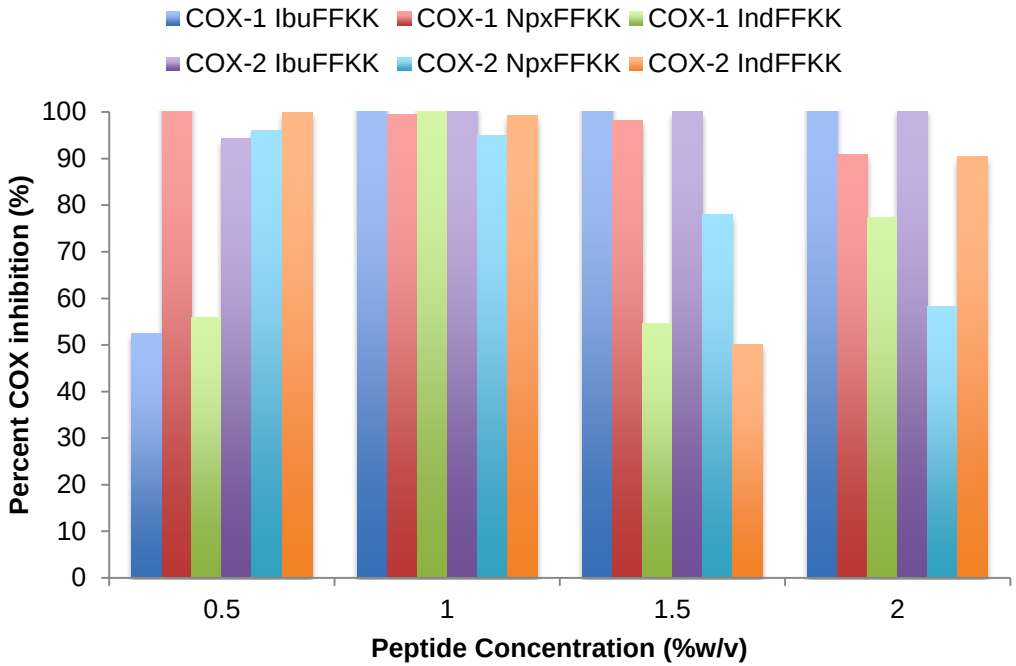
Dual action

Antimicrobial



Percentage reduction of mature 24h biofilm treated with 2% w/v NSAID- conjugated hydrogels utilizing an alamarBlue assay.

Anti-inflammatory



Percent inhibition of COX 1 and 2 enzyme by NSAID self-assembled hydrogels and by the model COX inhibitor DuP-697 using a COX Fluorescent Inhibitor Screening Assay Kit.

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Conclusion

- Developed a library of ultrashort self-assembling bifunctional peptides
- Vast potential for use against Biomaterial/Medical Device/Implant Infections
- Wound healing/surgical gel: Increased healing as mimics natural tissues
- Platforms/vehicles to deliver existing antimicrobials, extend spectrum of activity to Gram-negatives
- Translatable and economically friendly form of nanotechnology for patient benefit

Urinary catheter encrustation

Acknowledgements

- Dr Garry Laverty & Professor Brendan Gilmore
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